



SEQUENCE LISTING

<110> Human Genome Science, Inc.
Principia Pharmaceutical Corporation

<120> Albumin Fusion Proteins

<130> PF543

<140> 09/833,117

<141> 2001-04-12

<150> 60/229,358

<151> 2000-04-12

<150> 60/256,931

<151> 2000-12-21

<150> 60/199,384

<151> 2000-04-25

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<170> PatentIn Ver. 2.1

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<222> 1)..(19)

<223> invertase leader sequence

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<223> first 5 amino acids of mature human serum albumin

<400> 7

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Ile	Ser	Ala	Asp	Ala	His	Lys	Ser
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21

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 ctcttgtgtg catcgaagcc acag 24

<210> 11
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<212> DNA

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ac 62

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 fragments with non-cohesive ends.

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 gaa aat ttc aaa gcc ttg gtg ttg att gcc ttt gct cag tat ctt cag 96
 Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
 20 25 30
 cag tgt cca ttt gaa gat cat gta aaa tta gtg aat gaa gta act gaa 144
 Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
 35 40 45
 ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa 192
 Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
 50 55 60
 tca ctt cat acc ctt ttt gga gac aaa tta tgc aca gtt gca act ctt 240
 Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
 65 70 75 80
 cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct 288
 Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
 85 90 95

gag	aga	aat	gaa	tgc	ttc	ttg	caa	cac	aaa	gat	gac	aac	cca	aac	ctc	336
Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Asn	Leu	
			100					105					110			
ccc	cga	ttg	gtg	aga	cca	gag	gtt	gat	gtg	atg	tgc	act	gct	ttt	cat	384
Pro	Arg	Leu	Val	Arg	Pro	Glu	Val	Asp	Val	Met	Cys	Thr	Ala	Phe	His	
		115					120					125				
gac	aat	gaa	gag	aca	ttt	ttg	aaa	aaa	tac	tta	tat	gaa	att	gcc	aga	432
Asp	Asn	Glu	Glu	Thr	Phe	Leu	Lys	Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg	
	130					135					140					
aga	cat	cct	tac	ttt	tat	gcc	ccg	gaa	ctc	ctt	ttc	ttt	gct	aaa	agg	480
Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Phe	Phe	Ala	Lys	Arg	
145					150					155					160	
tat	aaa	gct	gct	ttt	aca	gaa	tgt	tgc	caa	gct	gct	gat	aaa	gct	gcc	528
Tyr	Lys	Ala	Ala	Phe	Thr	Glu	Cys	Cys	Gln	Ala	Ala	Asp	Lys	Ala	Ala	
				165				170						175		
tgc	ctg	ttg	cca	aag	ctc	gat	gaa	ctt	cgg	gat	gaa	ggg	aag	gct	tcg	576
Cys	Leu	Leu	Pro	Lys	Leu	Asp	Glu	Leu	Arg	Asp	Glu	Gly	Lys	Ala	Ser	
			180					185					190			
tct	gcc	aaa	cag	aga	ctc	aaa	tgt	gcc	agt	ctc	caa	aaa	ttt	gga	gaa	624
Ser	Ala	Lys	Gln	Arg	Leu	Lys	Cys	Ala	Ser	Leu	Gln	Lys	Phe	Gly	Glu	
		195					200					205				
aga	gct	ttc	aaa	gca	tgg	gca	gtg	gct	cgc	ctg	agc	cag	aga	ttt	ccc	672
Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg	Leu	Ser	Gln	Arg	Phe	Pro	
	210					215					220					
aaa	gct	gag	ttt	gca	gaa	gtt	tcc	aag	tta	gtg	aca	gat	ctt	acc	aaa	720
Lys	Ala	Glu	Phe	Ala	Glu	Val	Ser	Lys	Leu	Val	Thr	Asp	Leu	Thr	Lys	
225					230					235					240	
gtc	cac	acg	gaa	tgc	tgc	cat	gga	gat	ctg	ctt	gaa	tgt	gct	gat	gac	768
Val	His	Thr	Glu	Cys	Cys	His	Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	
				245				250						255		
agg	gcg	gac	ctt	gcc	aag	tat	atc	tgt	gaa	aat	cag	gat	tcg	atc	tcc	816
Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Ile	Cys	Glu	Asn	Gln	Asp	Ser	Ile	Ser	
			260					265					270			
agt	aaa	ctg	aag	gaa	tgc	tgt	gaa	aaa	cct	ctg	ttg	gaa	aaa	tcc	cac	864
Ser	Lys	Leu	Lys	Glu	Cys	Cys	Glu	Lys	Pro	Leu	Leu	Glu	Lys	Ser	His	
		275					280						285			

tgc att gcc gaa gtg gaa aat gat gag atg cct gct gac ttg cct tca	912
Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser	
290 295 300	
tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct	960
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala	
305 310 315 320	
gag gca aag gat gtc ttc ctg ggc atg ttt ttg tat gaa tat gca aga	1008
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg	
325 330 335	
agg cat cct gat tac tct gtc gtg ctg ctg ctg aga ctt gcc aag aca	1056
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr	
340 345 350	
tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa	1104
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu	
355 360 365	
tgc tat gcc aaa gtg ttc gat gaa ttt aaa cct ctt gtg gaa gag cct	1152
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro	
370 375 380	
cag aat tta atc aaa caa aac tgt gag ctt ttt gag cag ctt gga gag	1200
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu	
385 390 395 400	
tac aaa ttc cag aat gcg cta tta gtt cgt tac acc aag aaa gta ccc	1248
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro	
405 410 415	
caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa	1296
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys	
420 425 430	
gtg ggc agc aaa tgt tgt aaa cat cct gaa gca aaa aga atg ccc tgt	1344
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys	
435 440 445	
gca gaa gac tat cta tcc gtg gtc ctg aac cag tta tgt gtg ttg cat	1392
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His	
450 455 460	
gag aaa acg cca gta agt gac aga gtc aca aaa tgc tgc aca gag tcc	1440
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser	
465 470 475 480	
ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca	1488

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
 485 490 495
 tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat 1536
 Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
 500 505 510
 ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca 1584
 Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
 515 520 525
 ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg 1632
 Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
 530 535 540
 aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag 1680
 Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
 545 550 555 560
 gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt 1728
 Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
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 35 40 45
 Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
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 Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
 65 70 75 80

Arg	Glu	Thr	Tyr	Gly	Glu	Met	Ala	Asp	Cys	Cys	Ala	Lys	Gln	Glu	Pro	85	90	95
Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Asn	Leu	100	105	110
Pro	Arg	Leu	Val	Arg	Pro	Glu	Val	Asp	Val	Met	Cys	Thr	Ala	Phe	His	115	120	125
Asp	Asn	Glu	Glu	Thr	Phe	Leu	Lys	Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg	130	135	140
Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Phe	Phe	Ala	Lys	Arg	145	150	155
Tyr	Lys	Ala	Ala	Phe	Thr	Glu	Cys	Cys	Gln	Ala	Ala	Asp	Lys	Ala	Ala	165	170	175
Cys	Leu	Leu	Pro	Lys	Leu	Asp	Glu	Leu	Arg	Asp	Glu	Gly	Lys	Ala	Ser	180	185	190
Ser	Ala	Lys	Gln	Arg	Leu	Lys	Cys	Ala	Ser	Leu	Gln	Lys	Phe	Gly	Glu	195	200	205
Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg	Leu	Ser	Gln	Arg	Phe	Pro	210	215	220
Lys	Ala	Glu	Phe	Ala	Glu	Val	Ser	Lys	Leu	Val	Thr	Asp	Leu	Thr	Lys	225	230	235
Val	His	Thr	Glu	Cys	Cys	His	Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	245	250	255
Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Ile	Cys	Glu	Asn	Gln	Asp	Ser	Ile	Ser	260	265	270
Ser	Lys	Leu	Lys	Glu	Cys	Cys	Glu	Lys	Pro	Leu	Leu	Glu	Lys	Ser	His	275	280	285
Cys	Ile	Ala	Glu	Val	Glu	Asn	Asp	Glu	Met	Pro	Ala	Asp	Leu	Pro	Ser	290	295	300
Leu	Ala	Ala	Asp	Phe	Val	Glu	Ser	Lys	Asp	Val	Cys	Lys	Asn	Tyr	Ala	305	310	315
Glu	Ala	Lys	Asp	Val	Phe	Leu	Gly	Met	Phe	Leu	Tyr	Glu	Tyr	Ala	Arg	325	330	335
Arg	His	Pro	Asp	Tyr	Ser	Val	Val	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Thr			

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Tyr	Glu	Thr	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Ala	Ala	Asp	Pro	His	Glu		
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Cys	Tyr	Ala	Lys	Val	Phe	Asp	Glu	Phe	Lys	Pro	Leu	Val	Glu	Glu	Pro		
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Gln	Asn	Leu	Ile	Lys	Gln	Asn	Cys	Glu	Leu	Phe	Glu	Gln	Leu	Gly	Glu		
385					390					395					400		
Tyr	Lys	Phe	Gln	Asn	Ala	Leu	Leu	Val	Arg	Tyr	Thr	Lys	Lys	Val	Pro		
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Gln	Val	Ser	Thr	Pro	Thr	Leu	Val	Glu	Val	Ser	Arg	Asn	Leu	Gly	Lys		
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Val	Gly	Ser	Lys	Cys	Cys	Lys	His	Pro	Glu	Ala	Lys	Arg	Met	Pro	Cys		
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Ala	Glu	Asp	Tyr	Leu	Ser	Val	Val	Leu	Asn	Gln	Leu	Cys	Val	Leu	His		
	450					455					460						
Glu	Lys	Thr	Pro	Val	Ser	Asp	Arg	Val	Thr	Lys	Cys	Cys	Thr	Glu	Ser		
465					470				475						480		
Leu	Val	Asn	Arg	Arg	Pro	Cys	Phe	Ser	Ala	Leu	Glu	Val	Asp	Glu	Thr		
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Tyr	Val	Pro	Lys	Glu	Phe	Asn	Ala	Glu	Thr	Phe	Thr	Phe	His	Ala	Asp		
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Ile	Cys	Thr	Leu	Ser	Glu	Lys	Glu	Arg	Gln	Ile	Lys	Lys	Gln	Thr	Ala		
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Leu	Val	Glu	Leu	Val	Lys	His	Lys	Pro	Lys	Ala	Thr	Lys	Glu	Gln	Leu		
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Lys	Ala	Val	Met	Asp	Asp	Phe	Ala	Ala	Phe	Val	Glu	Lys	Cys	Cys	Lys		
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site in pPPC0006

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site in pPPC0006

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site in pPPC0006

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site in pPPC0006

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<212> DNA
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<223> Synthetic oligonucleotide used to alter restriction
sites in pPPC0007

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sites in pPPC0007

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agaattaagc ttagttttaa cggccggccg gcgcgcctta ttataagcct aaggcagctt 60

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<223> forward primer useful for generation of albumin
fusion protein in which the albumin moiety is N-terminal
of the Therapeutic Protein

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gcgcgcgttt aaacggccgg cggcgcgccc ttattannnn nnnnnnnnnn n

51

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<223> n equals a,t,g, or c

<400> 27
aggagcgtcg acaaaagann nnnnnnnnnn nnn

33

<210> 28
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for generation of albumin
fusion protein in which the albumin moiety is c-terminal of
the Therapeutic Protein

<220>
<221> misc_feature
<222> (38)
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<223> n equals a,t,g, or c

<400> 28
ctttaaatcg atgagcaacc tcactcttgt gtgcacnnn nnnnnnnnnn nn

52

<210> 29
 <211> 24
 <212> PRT
 <213> Artificial Sequence

 <220>
 <221> signal
 <223> signal peptide of natural human serum albumin protein

 <400> 29
 Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
 1 5 10 15

 Tyr Ser Arg Ser Leu Asp Lys Arg
 20

 <210> 30
 <211> 114
 <212> DNA
 <213> Artificial Sequence

 <220>
 <221> primer_bind
 <223> forward primer useful for generation of PC4:HSA
 albumin fusion VECTOR

 <220>
 <221> misc_feature
 <222> (5)..(10)
 <223> BamHI restriction site

 <220>
 <221> misc_feature
 <222> (11)..(16)
 <223> Hind III restriction site

 <220>
 <221> misc_feature
 <222> (17)..(27)
 <223> Kozak sequence

 <220>
 <221> misc_feature
 <222> (25)..(97)
 <223> cds natural signal sequence of human serum albumin

 <220>
 <221> misc_feature
 <222> (75)..(81)

<223> XhoI restriction site

<220>
 <221> misc_feature
 <222> (98)..(114)
 <223> cds first six amino acids of human serum albumin

<400> 30
 tcagggatcc aagcttccgc caccatgaag tgggtaacct ttatttcctc tctttttctc 60
 tttagctcgg cttactcgag ggggtgtgttt cgtcgagatg cacacaagag tgag 114

<210> 31
 <211> 43
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> primer_bind
 <223> reverse primer useful for generation of
 PC4:HSA albumin fusion VECTOR

<220>
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 <222> (6)..(11)
 <223> Asp718 restriction site

<220>
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 <222> (12)..(17)
 <223> EcoRI restriction site

<220>
 <221> misc_feature
 <222> (15)..(17)
 <223> reverse complement of stop codon

<220>
 <221> misc_feature
 <222> (18)..(25)
 <223> AscI restriction site

<220>
 <221> misc_feature
 <222> (18)..(43)
 <223> reverse complement of DNA sequence encoding last 9 amino acids

<400> 31

gcagcgggtac cgaattcggc ggcgccttata agcctaaggc agc

43

<210> 32

<211> 46

<212> DNA

<213> Artificial Sequence

<220>

<221> primer_bind

<223> forward primer useful for inserting Therapeutic protein into pC4:HSA vector

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<222> (29)

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<222> (30)

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<223> n equals a,t,g, or c

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<400> 32
ccgccgctcg aggggtgtgt ttcgtcgann nnnnnnnnnn nnnnnn

46

<210> 33
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for inserting Therapeutic
protein into pC4:HSA vector

<220>
<221> misc_feature
<222> (38)
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<221> misc_feature
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<222> (41)
<223> n equals a,t,g, or c

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<221> misc_feature
<222> (42)
<223> n equals a,t,g, or c

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<222> (43)
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<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

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<220>

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<221> misc_feature
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<220>
<221> misc_feature
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<400> 33
agtcccatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnnn nnnnn      55

<210> 34
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Stanniocalcin signal peptide

<400> 34
Met Leu Gln Asn Ser Ala Val Leu Leu Leu Leu Val Ile Ser Ala Ser
  1             5             10             15

Ala

<210> 35
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Synthetic signal peptide

<400> 35
Met Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Leu Ala Leu
  1             5             10             15

Trp Ala Pro Ala Arg Gly
          20

<210> 36
<211> 733
<212> DNA
<213> Homo sapiens

<400> 36

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gggatccgga gcccaaattct tctgacaaaa ctcacacatg cccaccgtgc ccagcacctg	60
aattcgaggg tgcaccgtca gtcttctctt tcccccaaaa acccaaggac accctcatga	120
tctcccggaac tcttgagggtc acatgcgtgg tgggtggacgt aagccacgaa gaccctgagg	180
tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca aagccgcggg	240
aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg caccaggact	300
ggctgaatgg caaggagtac aagtgcagg tctccaacaa agccctccca acccccatcg	360
agaaaacat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac accctgcccc	420
catcccgga tgagctgacc aagaaccagg tcagcctgac ctgcctgggtc aaaggcttct	480
atccaagcga catcgccgtg gagggggaga gcaatgggca gccggagaac aactacaaga	540
ccacgcctcc cgtgctggac tccgacggct ccttcttctt ctacagcaag ctcaccgtgg	600
acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat gaggctctgc	660
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<210> 37

<211> 5

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<223> membrane proximal motif of class 1 cytokine receptors

<220>

<221> misc_feature

<222> (3)

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<400> 37

Trp Ser Xaa Trp Ser

1

5

<210> 38

<211> 86

<212> DNA

<213> Artificial Sequence

<220>
 <221> primer_bind
 <223> forward primer useful for generation of a synthetic gamma activation site (GAS) containing promoter element

<400> 38
 gcgcctcgag atttccccga aatctagatt tccccgaaat gatttccccg aaatgatttc 60
 cccgaaatat ctgccatctc aattag 86

<210> 39
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> primer_bind
 <223> reverse primer useful for generation of a synthetic gamma activation site (GAS) containing promoter element

<400> 39
 gcggcaagct ttttgcaaag cctaggc 27

<210> 40
 <211> 271
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> misc_feature
 <223> Synthetic GAS-SV40 promoter sequence

<400> 40
 ctcgagattt ccccgaaatc tagatttccc cgaaatgatt tccccgaaat gatttccccg 60
 aaatatctgc catctcaatt agtcagcaac catagtcccc cccctaactc cgcccatccc 120
 gccctaact ccgcccagtt ccgcccattc tccgccccat ggctgactaa ttttttttat 180
 ttatgcagag gccgaggccg cctcggcctc tgagctattc cagaagtagt gaggaggctt 240
 ttttgagggc ctaggctttt gcaaaaagct t 271

<210> 41
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> primer_bind
 <223> primer useful for generation of a EGR/SEAP reporter construct

 <400> 41
 gcgctcgagg gatgacagcg atagaacccc gg 32

 <210> 42
 <211> 31
 <212> DNA
 <213> Artificial Sequence

 <220>
 <221> primer_bind
 <223> primer useful for generation of a EGR/SEAP reporter construct

 <400> 42
 gcgaagcttc gcgactcccc ggatccgcct c 31

 <210> 43
 <211> 12
 <212> DNA
 <213> Artificial Sequence

 <220>
 <221> misc_binding
 <223> NF-KB binding site

 <400> 43
 ggggactttc cc 12

 <210> 44
 <211> 73
 <212> DNA
 <213> Artificial Sequence

 <220>
 <221> primer_bind
 <223> forward primer useful for generation of a vector containing the
 NF-KB promoter element

 <400> 44
 gcggcctcga ggggactttc ccggggactt tccggggact ttccgggact ttccatcctg 60
 ccattctcaat tag 73

 <210> 45

<211> 256
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic NF-KB/SV40 promoter

<400> 45
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caattagtca gcaaccatag tcccggccct aactccgccc atcccgcccc taactccgcc 120
cagttccgcc cattctccgc cccatggctg actaattttt tttatttatg cagaggccga 180
ggccgcctcg gcctctgagc tattccagaa gtagtgagga ggcttttttg gaggcctagg 240
cttttgcaaa aagctt 256